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Aberrant Activity of TAK1 is Associated with Retinal Pathology

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Abstract

Transforming growth factor-β-activated kinase-1 (TAK1) is a mitogen activated protein kinase kinase kinase that is involved in diverse biological roles across species. Functioning downstream of TGF-β, TAK1 mediates the activation of the c-Jun N-terminal kinase (JNK) signaling pathway, serves as the target of pro-inflammatory cytokines, such as TNF-α, mediates NF-κβ activation, and plays a role in Wnt signaling in mesenchymal stem cells. Still, the expression of TAK1 in the retina has not been defined.

In our study, pathological and immunohistochemical assessments indicate a link between retinal pathology and TAK1 phosphorylation. We observed similar TAK1 expression both in non-obvious and obvious retinal pathologies. However, the phosphorylated form of TAK1 in the segments of retina with obvious pathology was hardly detected compared to its expression in the segments with non-obvious pathology. This finding indicates, for the first time, a possible involvement of TAK1 in human retinal pathologies. Better understanding the expression pattern of TAK1 may serve as a new therapeutic avenue for retinal pathologies.

Keywords: Transforming growth factor-β-activated kinase 1 (TAK1); Retinal pigment epithelium (RPE); Phosphorylation

Introduction

Transforming growth factor-β-activated kinase 1 (TAK1) is a MAP3K (MAP kinase kinase kinase) which activates the p38, JNK-MAPKs (c-Jun N-terminal protein kinase of mitogen-activated protein kinases) and NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) pathways following various stimulations [1-3]. TAK1 is activated downstream of several receptors and cascades, such as transforming growth factor-β (TGF-β), Toll-Like receptors, Interleukin-1 (IL-1), tumor necrosis factor alpha (TNF-α), T-cell receptor and B-cell receptor, all are inflammation-associated cytokines and chemokines [4]. Upon binding by their corresponding ligands, these receptors attract TNF receptor associated factors (TRAFs), which activate TAK1 [5].

TAK1 was originally identified as a MAPK kinase kinase 7 (MKKK7 or MAP3K7) in the TGF-β signaling pathway, which can also be activated by environmental stress. For TAK1 activation, phosphorylation at Thr-187 and Ser-192 residues in the activation loop of TAK1 is essentially required [6]. TAK1 transduces signals to several downstream signaling cascades, including the MAPK kinase (MKK) 4/7-JNK cascade, MKK3/6-p38 MAPK cascade and nuclear factor κB (NF-κB)-inducing kinase-IκB kinase cascade [7]. Recently, it has been shown that TAK1 is a major mediator of TGF-β1-induced type I collagen and fibronectin expression through activation of the MKK3-p38 MAPK and MKK4-JNK signaling cascades, respectively [8]. Furthermore, increased expression and activation of TAK1 enhances p38 phosphorylation and promotes interstitial fibrosis [9]. These data ascribe a crucial role to TAK1 in extracellular matrix production and tissue fibrosis. TAK1 is also implicated in regulation of cell cycle, cell apoptosis, inflammation, and in the Smad signaling pathway [10,11]. Thus, TAK1 may function as an important regulator and mediator of TGF-β1-induced Smad-dependent and Smad-independent signaling pathways.

Acting upstream in these crucial signal-transduction pathways, TAK1 has been the subject of numerous studies, both in vitro and in vivo. However, a comprehensive study of TAK1 function is challenging, since its ablation leads to embryonic lethality [12,13]. Studies using cell-type specific conditional knock-out mice revealed that TAK1 regulates inflammatory and fibrotic responses in skin and others tissues [14]. In addition, an inducible knock-out TAK1 deletion was shown to promote bone marrow and liver failures due to massive apoptotic cell-death of the bone marrow and hepatocytes [15].

In human pathology, TAK1 is often mentioned in the context of autoimmune diseases. Numerous publications have demonstrated a crucial role for TAK1 in pro-inflammatory signaling, such as those induced by TLRs, NOD2, IL-1β, TNF-α, T-cell and B-cell receptors [16,17]. In addition, previous studies performed in our laboratory have shown that TAK1 expression in human retinal pigment epithelial (RPE) cells was high and altered following oxidative stress. Moreover, TAK1 inhibition led to reduction in cell proliferation, cell-cycle arrest at G0/G1, and increased SA-β-gal expression, all known to be features of cell senescence [18]. Exposure of cells to oxidative stress combined with inhibition of TAK1 activity decreased the expression of pro-apoptotic proteins, such as p53, and promoted cellular senescence [18]. The study hereby aims to further question TAK1 pattern of expression in retinal pathologies by using specimens from enucleated eyes.

Materials and Methods

The study adhered to the tenets of the declaration of Helsinki and was performed in compliance with the Health Insurance Portability and Accountability Act. Institute: Sheba Medical Center, Permit ID number-SMC-13-0830.

Human retinal specimens were collected from enucleated eyes. The causes of enucleations were either blind painful eyes or intraocular tumors. The state of blind painful eyes resulted from numerous pathologies, including retinal ischemia. In contrast, intraocular tumors are usually isolated retinal findings which affect only well-defined retinal segments, leaving the unaffected retina healthy. Hence, tumor-free retinal segment from these eyes may represent healthy retina.

We defined the retinas taken from blind painful eyes as having obvious pathology, whereas the segments of retina with normal appearance, from eyes with intraocular tumors, were defined as specimens with non-obvious retinal pathology.

To study TAK1 possible involvement and to characterize its expression pattern in human retinal pathologies, we examined TAK1 expression and activity in both obvious and non-obvious retinal pathology specimens.

Immunohistochemistry

The specimens from either group were embedded in paraffin, and 4 µm sections were boiled for antigen retrieval (10 mM citrate buffer 20 min), rinsed in PBS, incubated for 1 hour with 0.2% tween and gelatin in PBS, washed with PBS, blocked with 2% normal goat serum in 1% BSA and 0.1% TritonX100 for 30 min and then incubated overnight with primary antibodies diluted in PBS. Antibodies used: rabbit polyclonal phospho-Thr187 TAK1 (Bioss, Woburn, MA, USA)[19]; rabbit polyclonal TAK1 (Novus, Littleton, CO, USA); Isotype control rabbit polyclonal. Following primary antibody reaction the slides were washed with PBS, incubated in H2O2 for 10 min, stained with Histofine Simple Stain TM MAX PO (Nichirei, Tokyo, Japan) for 10 min at room temperature (RT). Following additional washing in PBS, the sections were stained using HRP-streptavidin for 10 min at RT, washed with PBS and incubated at RT with diaminobenzidine for 10 min, washed and counterstained with hematoxylin for 2 min at RT, washed in H2O, dehydrated in graded alcohol (70-100%) and finally mounted on coverslips. Slides were photographed by Olympus bx51 microscope and and Cell^D software (Olympus, Modiin, Israel).

Quantification

TAK1 and phospho-TAK1 expressions were determined according to the intensity of the signal in the different retinal layers. Not detected, + small levels of expression, ++ medium levels of expression, +++ high levels of expression in all layers.

Results

Ten retinal specimens from ten enucleated eyed of ten patients were evaluated, five from blind painful eyes (representing obvious retinal pathology), and five from eyes with intraocular tumors (representing non-obvious retinal pathology). The age of the patients and their ocular clinical diagnosis are listed in Table 1.

Segment with obvious Retinal pathology			
Clinical diagnosis	Gender	Age	Patient
Blind, painful eye (clinical diagnosis unknown)	male	87	1
Blind Eye, Choroidal hemorrhage following traumatic perforation	female	82	2
Blind eye, neovascular glaucoma following CRVO*	male	72	3
Blind eye, Infectious choroiditis	male	85	4
Blind eye, Glaucoma and CRVO*	male	71	5
Segment with non-obvious Retinal pathology			
Clinical diagnosis	Gender	Age	Patient
Retinoblastoma	female	2	6
Orbital lymphangioma	female	10	7
Retinoblastoma	male	1	8
Invasive Basal Cell Carcinoma (BCC)	female	82	9
Malignant melanoma	female	71	10
*CRVO = central retinal vein occlusion ** BCC = Invasive Basal Cell Carcinoma			

Table 1: Characteristics of patients' enucleated eyes.

TAK1 expression in human retinal specimens

Several retinal pathologies induce aberrant activation of MAP kinase proteins [20]. However, very little is known about TAK1 involvement and expression in human pathologies. In this study we have characterized TAK1 pattern of expression in non-obvious pathological segments and in obvious pathological segments of the different retina.

TAK1 was found to be highly expressed in the outer nuclear layer, inner nuclear layer, ganglion cells, choroidal endothelial cells and RPE cells of all retinal specimens (Figure 1A and Table 2). In most cells TAK1 expression was localized mainly in the nucleus. This pattern of expression was demonstrated both in segments of non-obvious retinal pathologies and segments from obvious pathologic retina specimens (Figures 1A and 1B).

In contrast, the expression of activated TAK1, detected by phospho-Thr 187 TAK1 antibodies, was different between the obvious and non-obvious pathological specimens. The presence of activated TAK1 was found in all layers of the normal retina, with highest levels in the inner nuclear cells (Figure 1C). However, in the pathologial retina phospho-TAK1 was hardly detected (Figure 1D).

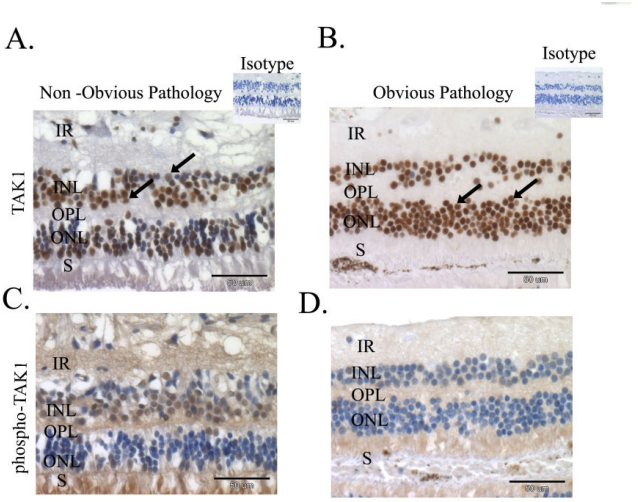

Figure 1: Aberrant Activity of TAK1 in Human Pathologic Retina. **A and B:** Retinal specimens, were immunostained with anti-TAK1 antibodies and hematoxylin (TAK1 expression is manifested by brown staining and black arrows), blue- hematoxylin. **C and D:** Retinal specimens were treated as in A and stained with phospho-Thr 187 TAK antibodies (brown) and hematoxylin (blue), demonstrating aberrant activity of TAK1 in the retina of blind painful eyes. IR-inner retinal layers; INL- inner nuclear layer; OPL- outer plexiform layer; ONL- outer nuclear layer; S- photoreceptor segment (Scale bar = 50μm).

TAK1 expression in the RPE cells

RPE cells play a key role in regulating the onset and progression of numerous retinal diseases, including age related macular degeneration (AMD) and proliferative vitreoretinopathy [21]. Consequently, we focused on the expression of TAK1 in the RPE cells. As can be seen in Figure 2A, in the healthy RPE cells the levels of TAK1 are moderate and mostly detected in the nucleus. Furthermore, the levels of phsopho-TAK1 are also mostly detected in the nucleus of the RPE cell. Interestingly, while TAK1 expression was obvious and seen in the pathological tissue (Figure 2B and 2C), activated TAK1 was not detected in the RPE cells of these specimens (Figure 2D).

The level of expression of TAK1 in its non-activated and activated states in each of the samples is shown in Table 2.

Segment with obvious Retinal pathology			
Phospho-TAK1 expression	TAK1 expression	Clinical diagnosis	Patient
Not detected	+++	Blind, painful eye (clinical diagnosis unknown)	1
Not detected	+++	Blind eye, Choroidal hemorrhage following traumatic perforation	2
+	+++	Blind eye, Neovascular glaucoma following CRVO*	3
Not detected	+++	Blind eye, Infectious choroiditis	4
Not detected	+++	Blind eye, Glaucoma and CRVO*	5
Segment with non-obvious Retinal pathology			
Phsopho-TAK1	TAK1 expression	Pathology Clinical diagnosis	Patient
+++	+++	Retinoblastoma	6
++	+++	Orbital lymphangioma	7
+++	+++	Retinoblastoma	8
+++	+++	Invasive BCC**	9
+++	+++	Malignant melanoma	10
*CRVO = central retinal vein occlusion ** BCC = Invasive Basal Cell Carcinoma			

Table 2: TAK1 and phospho-TAK1 expression in retinal specimens.

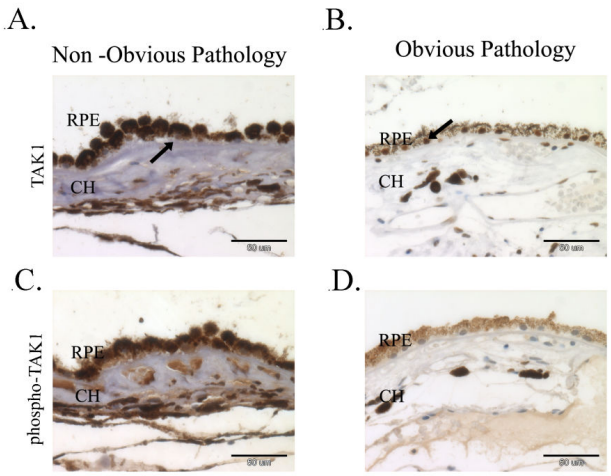

Figure 2: Attenuated TAK1 Activity in RPE Cells with Pathology. **A and B:** RPE cells of a retinal specimen were immunostained with TAK1 antibodies and hematoxylin. (TAK1 is manifested by brown staining and black arrows), blue- hematoxylin (N=5). **C and D:** Retinal specimens treated as in A and stained with phospho-Thr 187 TAK antibodies (brown) and hematoxylin (blue), demonstrating aberrant activity of TAK1 in the RPE cells of blind painful eyes (Scale bar = 50μm).

Discussion

This study defines for the first time the expression pattern of TAK1 MAP kinase in segments of human retina. Our data reveals TAK1 expression pattern in all human retina layers and demonstrates aberrant activity of TAK1 in human pathologic retinal segments.

TAK1 is a key player in the processes of inflammation and malignancy, regulating numerous downstream proteins [22]. Previous work has shown that TAK1 is expressed ubiquitously during early development of mice. During mid-gestation, TAK1 expression becomes more restricted, with high levels seen specifically during development of diverse organs and tissues, including the nervous system, testis, kidney, liver, gut, lung and pancreas [23], suggesting TAK1 may play multiple roles in mouse organ development. Of note, the expression of TAK1 detected in this study was mostly restricted to the cell nucleus. A recent publication indicated that TAK1 is expressed in the murine cochlea and that its expression pattern changes as the cochlea matures [24]. Whereas TAK1 was broadly expressed in both the developing otocyst and periotic mesenchyme in the murine embryo, by adulthood TAK1 labeling was limited to the pillar cells and the supporting cells in direct contact with inner and outer hair cells [24].

In contrast, very little is known regarding TAK1 expression in human cells. In human pathology, TAK1 is often mentioned in the context of autoimmune disease. [17]. In addition, TAK1 is involved in the activation of rheumatoid arthritis synoviocytes and human articular cartilage [25].

In the current study, the specimens of obvious pathologic retina were obtained from enucleated blind painful eyes. The causes for blind painful eye in these cases were diverse, including trauma, end stage glaucoma and retinal vein occlusion (as can be seen in Table 1). The specimens with presumed normal retina were obtained from segments of retina from eyes enucleated due to intraocular malignancy or orbital tumors. Segments of retina located away from the tumor were used; these segments are unaffected by the tumor and contain intact retina.

We found that the expression of TAK1 is manifested mainly in the nuclei of the outer nuclear layer, inner nuclear layer, ganglion cells, choroidal endothelial cells and RPE cells. Similar expression patterns were detected in both normal and pathologic retinas. It is well known that TAK1 activation is stimulated by phosphorylation on threonine 187 residues upon inflammatory response, stress or malignancy [6]. Therefore, this study examined the activity of TAK1 utilizing specific antibodies recognizing the phosphorylation on Thr-187 residue. The data presented hereby reveals that while TAK1 is present in both normal and pathologic retinas, its activity in the obvious pathological retina is barely detected in the outer nuclear layer, inner nuclear layer, ganglion cells and choroidal endothelial cells, and is further diminished in the RPE cells.

Our findings regarding TAK1 expression and activity in the human retina place it as a key factor in retinal pathologies. Even though there is diversity in the etiology of the different cases with blind painful eye, the same pattern of expression in the activated form of TAK1 was observed. We suggest that the retinal pathology may attenuate TAK1 activity, thus contributing to the deterioration of the retina; however, we cannot rule out the possibility that aberrant activity of TAK1 contributes to the onset and/or the progression of retinal pathologies. Still, these assumptions need to be further studied to reveal the exact role of TAK1 in retinal pathophysiologies.

In conclusion, this study demonstrates for the first time the pattern of expression of TAK1 in the human retina and characterizes its activity expression in the human pathologic retina. Nevertheless, additional study is required in order to delineate the mechanism by which TAK1 participates in the different retinal pathologic disorders. Elucidating the role of TAK1 in the human retina and other ocular tissues may be used as a potential therapeutic avenue for ocular disorders.

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References

1. Kim SI, Kwak JH, Zachariah M, He Y, Wang L, et al. (2007) TGF-beta-activated kinase 1 and TAK1-binding protein 1 cooperate to mediate TGF-beta1-induced MKK3-p38 MAPK activation and stimulation of type I collagen. Am J Physiol Renal Physiol 292: F1471-1478.
2. Mu Y, Gudey SK, Landstrom M (2011) Non-Smad signaling pathways. Cell Tissue Res 347: 11-20.
3. Hanafusa H, Ninomiya-Tsuji J, Masuyama N, Nishita M, Fujisawa J, et al. (1999) Involvement of the p38 mitogen-activated protein kinase pathway in transforming growth factor-beta-induced gene expression. J Biol Chem 274: 27161-27167.
4. Kim SI, Kwak JH, Na HJ, Kim JK, Ding Y, et al. (2009) Transforming growth factor-beta (TGF-beta1) activates TAK1 via TAB1-mediated autophosphorylation, independent of TGF-beta receptor kinase activity in mesangial cells. J Biol Chem 284: 22285-22296.
5. Tseng PH, Matsuzawa A, Zhang W, Mino T, Vignali DA, et al. (2010) Different modes of ubiquitination of the adaptor TRAF3 selectively activate the expression of type I interferons and proinflammatory cytokines. Nat Immunol 11: 70-75.
6. Singhirunnusorn P, Suzuki S, Kawasaki N, Saiki I, Sakurai H (2005) Critical roles of threonine 187 phosphorylation in cellular stress-induced rapid and transient activation of transforming growth factor-beta-activated kinase 1 (TAK1) in a signaling complex containing TAK1-binding protein TAB1 and TAB2. J Biol Chem 280: 7359-7368.
7. Lee J, Mira-Arbibe L, Ulevitch RJ (2000) TAK1 regulates multiple protein kinase cascades activated by bacterial lipopolysaccharide. J Leukoc Biol 68: 909-915.
8. Choi ME, Ding Y, Kim SI (2012) TGF-beta signaling via TAK1 pathway: role in kidney fibrosis. Semin Nephrol 32: 244-252.

9. Zhang D, Gaussin V, Taffet GE, Belaguli NS, Yamada M, et al. (2000) TAK1 is activated in the myocardium after pressure overload and is sufficient to provoke heart failure in transgenic mice. Nat Med 6: 556-563.
10. Hoffmann A, Preobrazhenska O, Wodarczyk C, Medler Y, Winkel A, et al. (2005) Transforming growth factor-beta-activated kinase-1 (TAK1), a MAP3K, interacts with Smad proteins and interferes with osteogenesis in murine mesenchymal progenitors. J Biol Chem 280: 27271-27283.
11. Dowdy SC, Mariani A, Janknecht R (2003) HER2/Neu- and TAK1-mediated up-regulation of the transforming growth factor beta inhibitor Smad7 via the ETS protein ER81. J Biol Chem 278: 44377-44384.
12. Sato S, Sanjo H, Takeda K, Ninomiya-Tsuji J, Yamamoto M, et al. (2005) Essential function for the kinase TAK1 in innate and adaptive immune responses. Nat Immunol 6: 1087-1095.
13. Jadrich JL, O'Connor MB, Coucouvanis E (2006) The TGF beta activated kinase TAK1 regulates vascular development in vivo. Development 133: 1529-1541.
14. Omori E, Matsumoto K, Sanjo H, Sato S, Akira S, et al. (2006) TAK1 is a master regulator of epidermal homeostasis involving skin inflammation and apoptosis. J Biol Chem 281: 19610-19617.
15. Tang M, Wei X, Guo Y, Breslin P, Zhang S, et al. (2008) TAK1 is required for the survival of hematopoietic cells and hepatocytes in mice. J Exp Med 205: 1611-1619.
16. Brown J, Wang H, Hajishengallis GN, Martin M (2011) TLR-signaling networks: an integration of adaptor molecules, kinases, and cross-talk. J Dent Res 90: 417-427.
17. Landstrom M (2010) The TAK1-TRAF6 signalling pathway. Int J Biochem Cell Biol 42: 585-589.
18. Dvashi Z, Green Y, Pollack A (2014) TAK1 Inhibition accelerates cellular senescence of retinal pigment epithelial cells. Invest Ophthalmol Vis Sci 55: 5679-5686.
19. Dvashi Z, Goldberg M, Adir O, Shapira M, Pollack A (2015) TGF-beta1 induced transdifferentiation of rpe cells is mediated by TAK1. PLoS One 10: e0122229.
20. Hecquet C, Lefevre G, Valtink M, Engelmann K, Mascarelli F (2003) Activation and role of MAP kinase-dependent pathways in retinal pigment epithelium cells: JNK1, P38 kinase, and cell death. Invest Ophthalmol Vis Sci 44: 1320-1329.
21. Hiscott P, Sheridan C, Magee RM, Grierson I (1999) Matrix and the retinal pigment epithelium in proliferative retinal disease. Prog Retin Eye Res 18: 167-190.
22. Sakurai H (2012) Targeting of TAK1 in inflammatory disorders and cancer. Trends Pharmacol Sci 33: 522-530.
23. Jadrich JL, O'Connor MB, Coucouvanis E (2003) Expression of TAK1, a mediator of TGF-beta and BMP signaling, during mouse embryonic development. Gene Expr Patterns 3: 131-134.
24. Parker MA, Jiang K, Kempfle JS, Mizutari K, Simmons CL, et al. (2011) TAK1 expression in the cochlea: a specific marker for adult supporting cells. J Assoc Res Otolaryngol 12: 471-483.
25. Hammaker DR, Boyle DL, Inoue T, Firestein GS (2007) Regulation of the JNK pathway by TGF-beta activated kinase 1 in rheumatoid arthritis synoviocytes. Arthritis Res Ther 9: R57.

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